

**THE EFFECTS OF COPPER
AND DEPRESSED pH
ON FLAGFISH REPRODUCTION**

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**Ministry
of the
Environment**

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THE EFFECTS OF COPPER AND DEPRESSED
pH ON FLAGFISH REPRODUCTION

by

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INTRODUCTION

Many lakes in the vicinity of Sudbury, Ontario, have been found to be extremely acidic (Beamish and Harvey 1972). Studies by Beamish and Van Loon (1977) showed that the pH of a lake (Lumsden) in the area was closely related to the measured amount of acid entering the lake from bulk precipitation. They also concluded that atmospheric fall-out contributed substantial quantities of nickel and copper to the watershed, some of which appeared to be retained in the lake water. Conroy et al. (1975), Costescu and Hutchinson (1972), Hutchinson and Whitley (1974) and Whitley and Hutchinson (1974) established that an indirect relationship existed between heavy metal loadings in soil and water with the distances from the smelters.

The effects of low pH waters on the physiology of freshwater fish has been investigated by many authors. Mount (1973) as well as Johansson et al. (1973) demonstrated that both egg production and hatchability were reduced in fathead minnows and zebra fish. Suckers exposed to low pH waters exhibited a reduced feeding response (Beamish 1972). Changes in several blood parameters occurred when rainbow trout were exposed to low pH waters (Jonas et al. 1962). Investigations by Packer and Dunson (1970 and 1972) determined that one of the direct effects of low pH on fish was the impairment of active gas transport through enzyme inhibition at the gill surface, Craig and Baksi (1977) indicated that egg production, fertility, fry survival and growth of flagfish were impaired at pH 6.5 or less, the most sensitive stage being egg production.

This study was designed to determine the combined effect of reduced pH and copper on flagfish reproduction. Data from Sudbury acid lakes studies (Beamish, 1973) indicated that where high acid and metal concentrations existed, fisheries were reduced. Copper was chosen as it is the most toxic metal emitted from the smelter stacks (iron, nickel, copper, lead) and is commonly found in high concentrations (<.002 - 2.5 ppm) in nearby Sudbury lakes (Yan et al. 1977). The level of pH depression was based on the previous study (Craig and Baksi, 1977)

METHODS

The study was conducted from May to September, 1975 in a mobile laboratory located on the shores of Lake Panache, approximately 20 Km west of Sudbury, Ontario. Water pumped from the lake was preheated to approximately 26°C in a head tank, which subsequently supplied a modified Mount-Brungs diluter (Mount and Brungs, 1967). Water temperature of the head tank was maintained by a 3000W chromalux heater. Final temperature control was furnished by 100 watt immersion heaters in the exposure tanks. No cooling system was required as the incoming water never exceeded 26°C. Copper sulphate (CuSO_4) was dosed at a single concentration in each of two runs ($7 \mu\text{g l}^{-1}$, and $14 \mu\text{g l}^{-1}$) across a pH range from 6.8 (control) to 5.5. The exposure regime consisted of four control tanks (no copper or acid), four control (pH 6.8) plus copper, duplicate pH 6.0 plus copper and duplicate pH 5.5 plus copper.

The lake water pH was adjusted in the diluter by the addition of 0.1 N sulphuric acid stock, injected by an Oxford automatic pipettor. A Leeds and Northrop pH controller-monitor continuously recorded the pH in the lowest pH exposure tanks providing a fail safe function and maintaining pH within 0.1 units of nominal levels. Copper was added to the required tanks by pumping a stock CuSO_4 solution to a mini-diluter (Fig. 1) from a large reservoir. The mini-diluter discharged the CuSO_4 solution directly to the final splitter-cells during the addition of the pH adjusted water from the primary and intermediate mixing-splitter cells. Copper was maintained at $7 (\sigma = .69)$ and $14 \mu\text{g l}^{-1} (\sigma = .005)$ above background.

The flagfish (Jordanella floridae) an oviparous species was chosen for its ease of culture and short life cycle. Fish were allowed to acclimate in lake water (Lake Panache) for a minimum of 14 days prior to use and were fed, chopped, rinsed earthworms and frozen brine shrimp four times daily. Two males and six female flagfish were randomly selected and placed in each of the exposure tanks. Control water was allowed to flow through the tanks until the number of eggs laid per tank was stable for a period of 13 days. Mortalities during the first 13 days were replaced, however, during the exposure period mortalities were not replaced. Tanks were cleaned daily. The pH, D.O. and temperature were recorded daily.

The pH and copper levels in the exposure tanks were altered linearly to the prescribed levels over five days. Control tanks received neither acid nor copper. Two bioassays were conducted, one at each copper level of 7 and 14 $\mu\text{g l}^{-1}$ with exposure times of 14 and 15 days respectively. Exposure duration was determined by the time required to demonstrate a measurable effect on egg production.

Water samples were collected daily in 100 ml polyethylene centrifuge tubes from the exposure tanks and analyzed for total copper. Analysis of treated exposure water for acidity, alkalinity, hardness, sulphate and conductivity was also complete. Background lake water quality was determined for metals, nutrients and dissolved solids.

RESULTS AND DISCUSSIONS

Studies by Mount (1968) and by Mount and Stephan (1969) indicate that the effects of copper on reproduction growth and survival of fathead minnows is more pronounced in soft water, as less CaCO_3 is available for complexation of the metal. Furthermore, data by Bandt (1946) and Doudoroff (1952) indicates that synergistic toxicity of metals would be expected, especially in soft water, and therefore though the concentrations of other metals present in Lake Panache were low (Table 1) a metals affect may exist.

All exposure groups were actively spawning and equally productive before pH and copper levels were altered (Table 3).

Flagfish spawning and egg production ceased in both 7 and 14 $\mu\text{g l}^{-1}$ copper exposure tanks within four days after acid and copper were introduced (Figs. 2 and 3). The 7 $\mu\text{g l}^{-1}$ group registered three spawning events after acid and copper addition and these were within the first three days of the 15 day exposure period. Control groups continued to spawn throughout the exposure period and mean daily output of eggs (Table 3) were comparable with the previous study (Craig and Baksi, 1977).

The limits of analytical detection for copper by A.A.S. ($\pm 2-3 \mu\text{g l}^{-1}$) did not allow the exposure of an intermediate concentration between 0 and 7 $\mu\text{g l}^{-1}$ above background. Consequently, the inhibition of egg production was essentially quantal from 0 to 100% between control and the lowest copper concentration (Fig. 2).

The rapid inhibition of egg production by copper under control pH conditions indicates the greater potency of copper, over acid, on an essential phase of the reproductive cycle.

Mortalities observed (up to 21%) during the exposure regimes were significant (Table 3). Though no direct cause can be ascertained, the combined effect of the acid-copper stress on a marginally healthy population could be a possible reason for some of the observed mortality. The significance of such an acid-copper stress to a population of questionable health may well be important as low level disease is endemic in the wild, and reproductive functions would then be more seriously affected. Regardless, the effect of the acid-copper on egg production is dramatic (Table 3).

SUMMARY AND CONCLUSIONS

Flagfish egg production was totally inhibited within three days of exposure to copper under control and pH depressed conditions (pH 5.5 - 6.8). The copper effect was more pronounced than the pH effect impairing reproduction at concentrations of 7 µg/l above a background level of 7.3 ppb

The study indicates how sublethal quantities of metals and acid can adversely effect aquatic life by subtly inhibiting reproductive ability.

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Table 1: Total Metal Concentrations (ppb) of Lake Panache Water
(Average of two analyses).

Metal	Concentration ($\mu\text{g l}^{-1}$)
Copper	7.3
Nickel	3.3
Lead	<3.0
Zinc	1.2
Cadmium	<1.0
Chromium	<20.0
Aluminum	<40.0
Manganese	3.0
Iron	1.6
Magnesium	2,300.0

Table 2: Water Quality Parameters (ppm) of Lake Panache Water
(Average of two analyses)

Constituent	Concentration (mg l ⁻¹)
Alkalinity	11.23
Hardness	28.0
Conductivity	81.5 μ mhos/cm
Sulphate	23.13
Free Ammonia	0.13
Nitrites	0.004
Nitrates	0.06
Kjeldahl	0.52
Total Phosphorus	0.25
pH	6.88

Table 3: Percent mortality and mean daily egg production on flagfish before and during exposure to nominal pH plus copper levels

	7 $\mu\text{g l}^{-1}$ Cu^{+2}				14 $\mu\text{g l}^{-1}$ Cu^{+2}			
	Mortalities		Egg Production		Mortalities		Egg Production	
	Before	During	Before	During	Before	During	Before	During
Control	6.25%	15.6%	59	60	3%	0%	31	78
Control + Cu^{+2}	6.25%	6.25%	69	0.6	9.3%	8%	36	0
pH 6 + Cu^{+2}	6.25%	18.75%	73	0.3	6.25%	6.25%	21	0
pH 5.5 + Cu^{+2}	0%	6.25%	90	0.0	12.5%	21.6%	47	0

Fig. 1. Schematic Cross-Section of mini-diluter

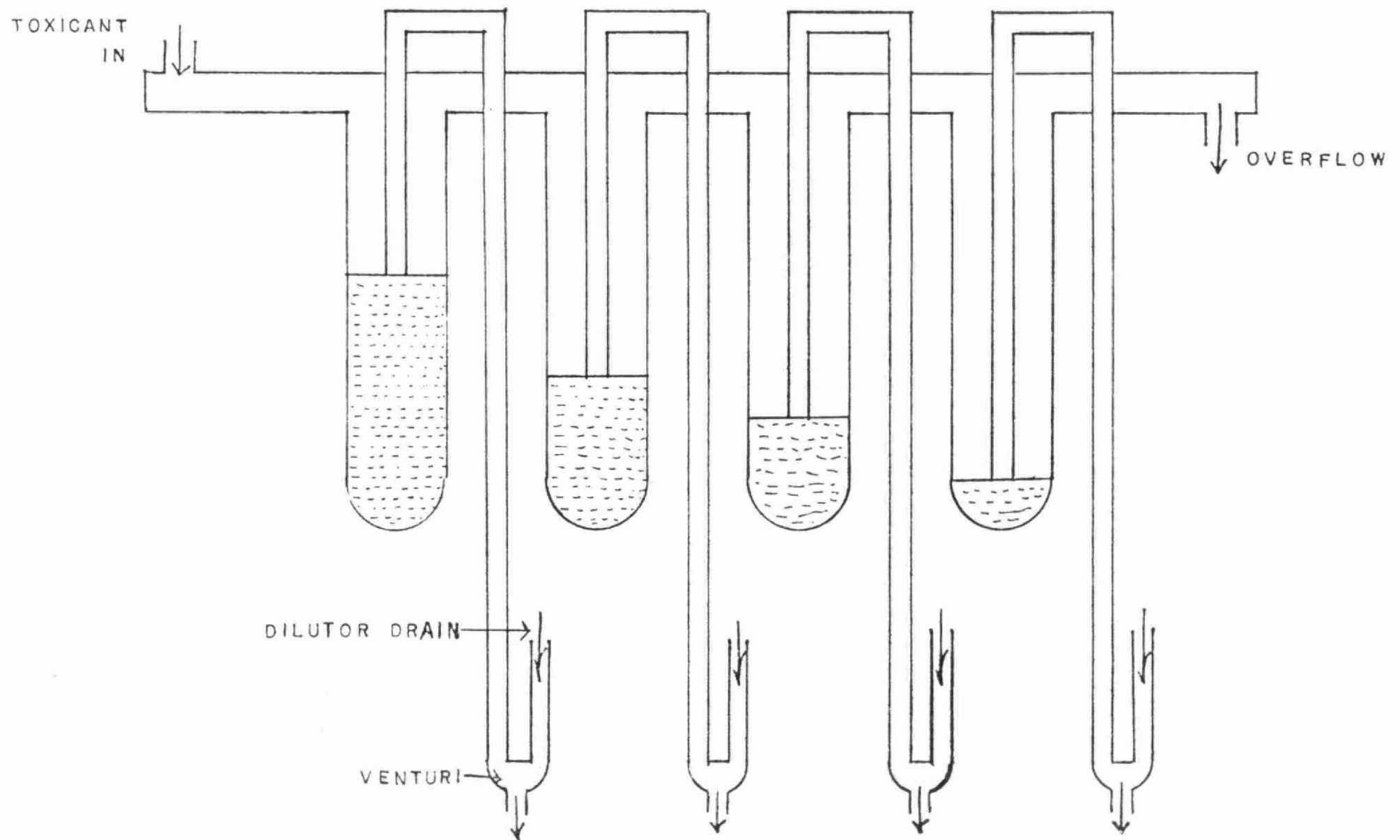


Fig. 2. Daily egg production of Flagfish during initial exposure to untreated lake water (pH 6.8), linear acid and copper addition and exposure to nominal acid and copper at $7 \mu\text{g l}^{-1}$

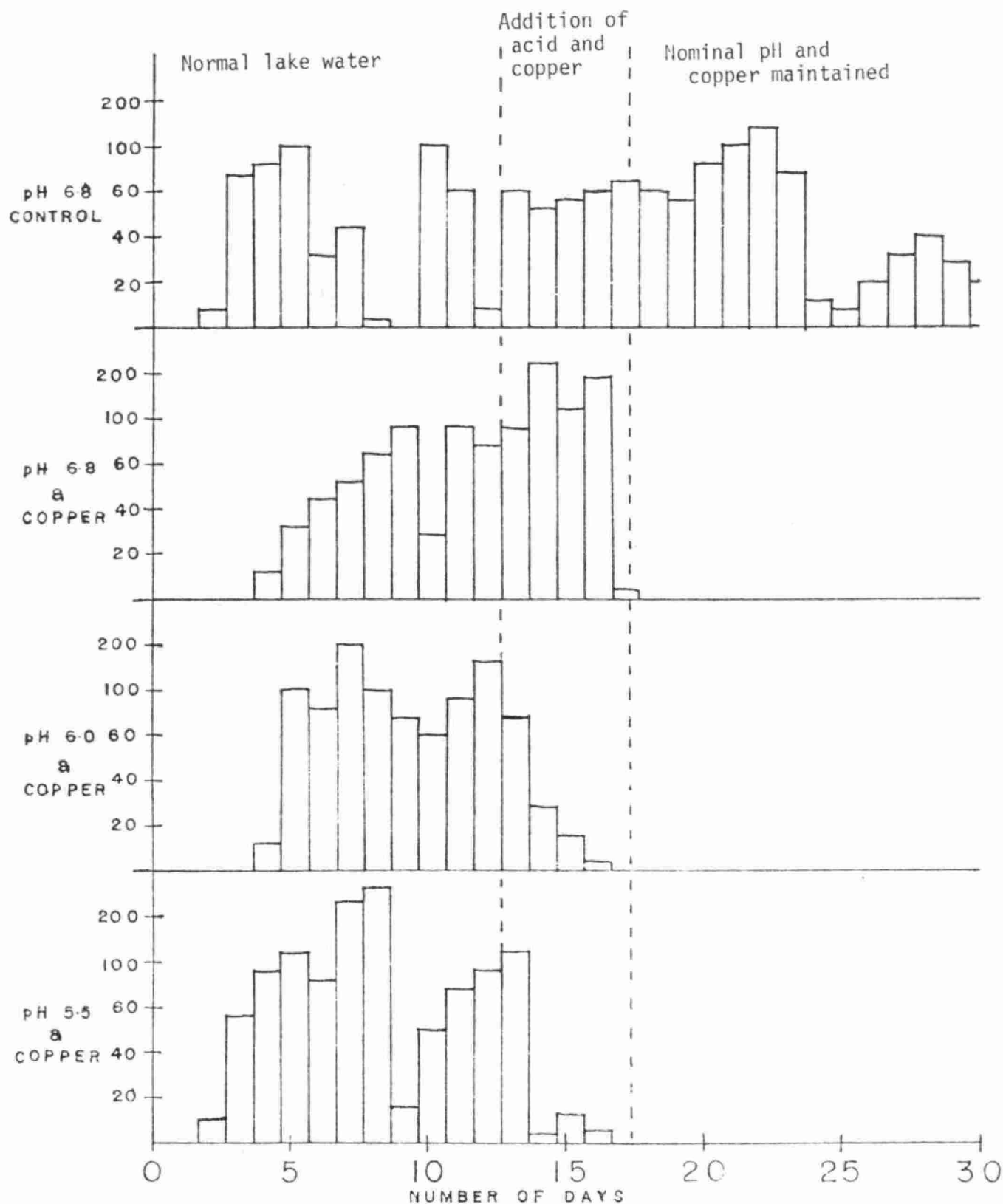
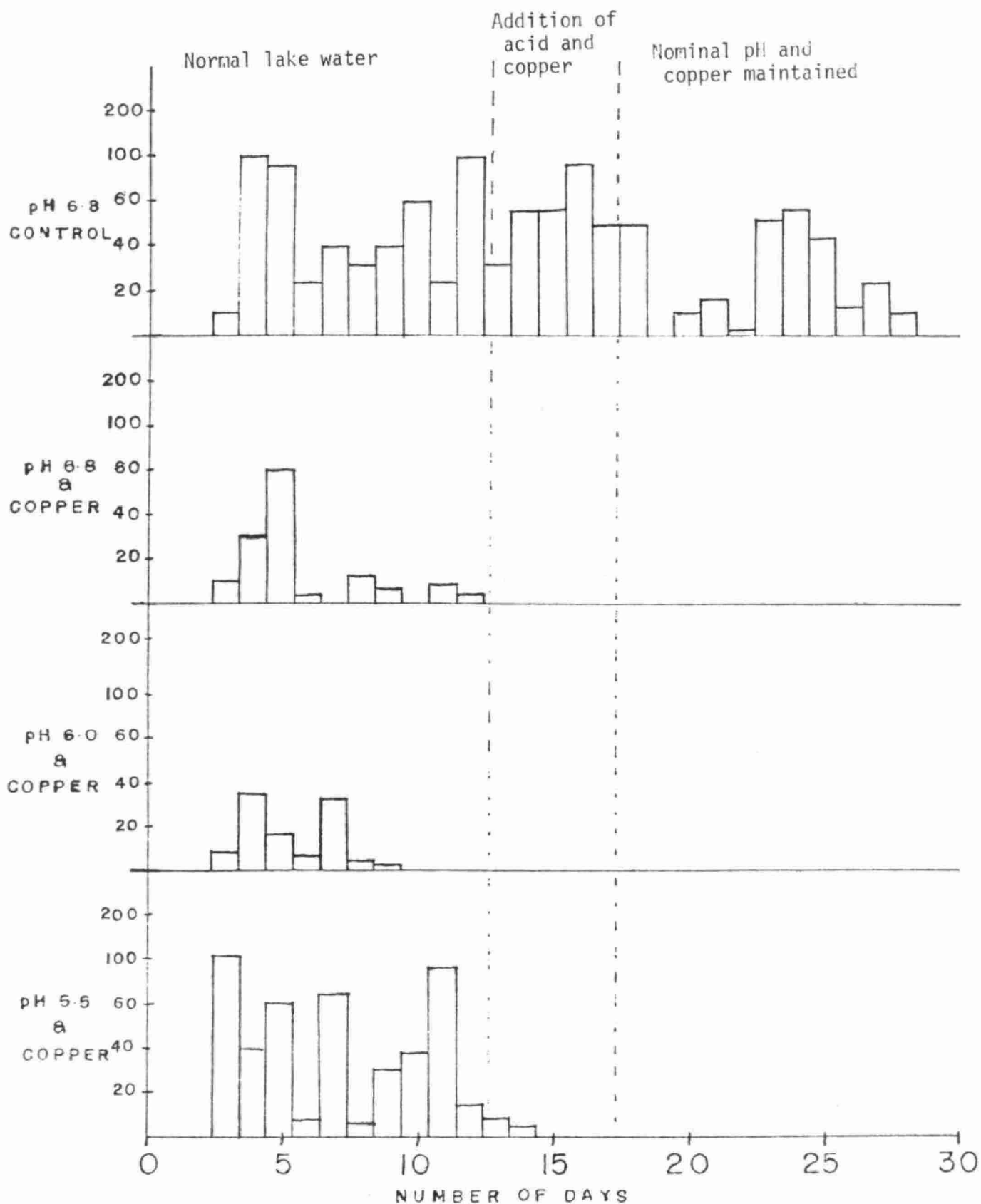


Fig. 3. Daily egg production of Flagfish during initial exposure to untreated lake water (pH 6.8) linear acid and copper addition and exposure to nominal acid and copper at $14 \mu\text{g l}^{-1}$.





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copper and depressed alqh

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